

TABLE 3: Prevention of Cooling Injury by Cryoprotective Polymers

	Cryoprotectant and Temperature*	Tonicity	K/Na	SEM	% of Control
5	Untreated controls in LM5, 0°C	1.0X	7.19	0.14	100
	Veg - 3% D(1)F in 1X LM5, 0°	1.0X		6.88 0.16	96
	Veg - 3% D(1)F in 1.5X LM5, 0°	1.5X		6.58 0.16	91.5
10	Veg - 3% D(1)F plus 0.7X polymers, at 0°C (in 1X LM5)	1.7X		6.92 0.16	96.3
	Veg - 3% D(1)F in 1.5X LM5, transferred to same at -20°C	1.5X		7.31 0.21	102
15	Veg - 3% D(1)F in 1X LM5 plus 0.7X polymers, transferred to same at -20°C	1.7X		6.48 0.14	90.1

* Veg - 3% D(1)F = 16.84 % w/v ethylene glycol plus 12.86% w/v formamide plus 22.3% w/v DMSO. 0.7X polymers are 7% w/v PVP of mean molecular weight 5,000 ("PVP 5000") plus 1% w/v X1000 (a polyvinyl alcohol product commercially available from 21st Century Medicine, 10844 Edison Court, Rancho Cucamonga, CA 91730; <http://www.21CM.com>), and 1% w/v decaglycerol. This was experiment 00-025. All solutions exposed for 20 min at 0°C prior to cooling, and after cooling were held in the same solutions for an additional 20 min. Groups consist of 12 samples each. Hypertonicity was present in the full-strength cryoprotectant only, other solutions being isotonic. PVP 5000 is a particularly effective polymer for promoting vitrification, and both X1000 and decaglycerol are antinucleators useful for the promotion of vitrification.

TABLE 4: Some Useful Reference Osmolalities and Tonicities

	Solution	Osmolality	Tonicity (X)
30	LM5	285 +/- 3 mOsm	1.0
	LM5 + 1% w/v decaglycerol	319 mOsm	1.12
	LM5 + 7% w/v PVP 5,000	417 mOsm	1.46
	LM5 + 7% w/v PVP 5,000	478 mOsm	1.68
35	+ 1% w/v X1000		
	+ 1% w/v decaglycerol		
	LM5 + 1% w/v X1000 +	346 mOsm (calculated)	1.21 (calculated)
	1% w/v decaglycerol		

*The composition of LM5 is disclosed in U.S. Patent Application No. 09/916,386, "Particularly Advantageous Cryoprotectant and Carrier Solution Compositions", Fahy *et al.*, filed July 26, 2001, herein incorporated by reference (from provisional application 60/221,691, filed July 31, 2000). *now U.S. Patent No. 6,869,757*.

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